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Draft SOP for Sample Matching using ICP-MS

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Introduction and Purpose

The purpose of this standard operating procedure (SOP) is to provide an assay for testing the hypothesis that two samples are from the same sub-population, which can mean they came from the same batch, set of replicate batches, or the same process[1]. The outcome is a likelihood ratio, which should be used in combination with prior odds to determine the posterior odds of the two samples coming from the same sub-population. Note that the term “match” is not used because it is imprecise in the context of determining the relationship between two samples.

Under this SOP, inductively coupled plasma mass spectrometer (ICP-MS) is used to generate elemental composition data, which is used to test the hypothesis that two samples come from the same sub-population. ICP-MS is a highly sensitive bulk analysis method. The quantity of sample required is ultimately determined by the instrumentation used and the elements selected for generating ROC curves. This SOP specifies that greater than 5 mg of sample be used to enable accurate weights to be obtained for the sample in tared 3 mL Teflon vials. However, we have conducted preliminary assays with our instrumentation that suggest that sample quantity can be reduced to 50 micrograms if elemental abundances are normalized to total metal content (see Step 10). We use ICP-MS because it is the most sensitive method for determining the concentration of the largest number of elements. Other elemental analysis methods could be used to generate data for this test (e.g., ICP-OES), but specific tests would be necessary to work out the SOP.

Personnel

Personnel who carry out steps 1 through 8 must be knowledgeable of both safe microbiology and wet bench laboratory procedures. Personnel who carry out steps 9 and 10 must be knowledgeable of ICP-MS operation and data reduction. Personnel who carry out steps 11 and 12 must be knowledgeable of the statistical methods in the accompanying report[2].

Materials

3 mL Teflon vial with screw top lid (purchased from Savillex #201-003-20-023-01)
Seastar® ultra high purity concentrated nitric acid (Seastar)
18.2 Mega Ohm water (deionized water)

10 mL polypropylene tubes (purchased through Evergreen Scientific through Fisher Scientific)
ICP-MS 5 ppb internal standard mix (purchased from Inorganic Ventures #IV-ICPMS-71D, Multi-element mix of ^{6}Li, Sc, In, and Bi)

Equipment

Analytical balance (0.1 mg precision or better)
MARS microwave digestion system (http://cem.com/page105.html).
Inductively coupled plasma mass spectrometer (ICP-MS)

CAUTION: For standard ICP-MS laboratories, pathogenic samples must be sterilized prior to handling. Irradiation is recommended.

1. **Assess sample for contamination and quantity**. Sample contamination is assessed because this is a bulk analysis method, and therefore, for this analysis to be meaningful, the sample must be reasonably uncontaminated with other material, or it must be sufficiently well characterized to allow deconvolution of the elemental data. Because the production method can result in heterogeneous material, it is necessary to consult with the source of a sample to determine if contamination is likely. Potentially appropriate samples include vials or packets of material and residues from production equipment. Characterization of sample physical appearance is beyond the scope of this SOP.

   Sample quantity is assessed to determine if the sample can be weighed and if this method is viable for the sample. If sufficient material is available, it is preferable to weigh out the material to enable absolution quantification of elemental abundances. Sample retention in the container must be considered. Sample quantity can be estimated based on the difference between the sample container and a comparable empty container. For smaller quantities, it may be necessary to use a visual assessment of the mass based on known masses of a comparable sample in a similar container. The starting mass should be at least 5 mg to successfully obtain a reliable mass for the sample and to provide sufficient material for high sensitivity elemental detection and measurement. (Note: Microbalances are outside the scope of this SOP. They can enable smaller masses to be quantified, but special procedures are necessary to prevent loss during transfer.)

   If the sample is contaminated or there is insufficient material for weighing, spatially resolved methods (e.g., secondary ion mass spectrometry, X-ray analysis) should be considered.

2. **Vial preparation.** Sample digestion is performed in 3 mL Teflon vials. The vials must first be cleaned by immersing in an 8M nitric acid solution (Seastar®, ultra high purity) and heated at 100°C for at least 24 hours. After soaking in nitric, the

vials should be heated at 100°C in 18.2 Mega Ohm water for at least 24 hours. Finally after heating, they should be rinsed three more times with water and placed in an oven at 60°C to dry. The vials should be checked by running several blanks through the digestion and analysis process (Steps 4 - 10) to verify that they are clean.

3. **Weigh sample.** A clean, dry 3 mL Teflon vial (no lid) should be weighed and tared. Sample should be either transferred by tapping the sample tube allowing the spore sample to fall into the teflon vials, or by using a metal spatula. The sample should be clean before and after use. To enable absolute quantification, greater than 5 mg of sample should be added to the vial. All digits on the scale display should be recorded.

4. **Acidify sample and make blanks.** After the dried sample is added to each vial, 1 mL of concentrated nitric acid (Seastar, ultra high purity) should be added. Three vials should be used as microwave digest blanks containing only the nitric acid. After the sample and nitric acid is added, the lid is screwed onto the vial and tightened by torque wrench (20 in lbs). This ensures that nothing will evaporate while digesting.

   Note: While this SOP does not detail methods for samples with insufficient material for weighing, the following options are offered as a guide. For water tight, acid proof containers, the acids can be directly added to the container to maximize sample recovery. For small containers (e.g., 1.5 mL vials), small aliquots of acid can be successively added and poured into a 3 mL Teflon vial to wash the sample from the container. For larger containers, more acid and/or deionized water can be used to maximize recovery; the sample can then be concentrated by evaporation prior to digestion. If the container is not acid proof, deionized water can be used to wash the sample into another container for concentrating and acidification. Wipes or other materials can be immersed in acid or deionzied water to recover the sample. Sample concentration may be necessary for these samples prior to acidification and digestion. If possible, the wipe or other material should be tested to determine how much material is washed from them.

   Note: The quantity of acid and deionized water should be minimized to keep the background low. Trace quantities of elements are present, even in high purity acid and water.

5. **Make control sample.** The digestion procedure is monitored by use of a control sample in a vial with a probe. An additional aliquot of one of the samples to be test or a comparable sample should be used for the control. The sample should be prepared using the same procedures in Steps 3 and 4. This sample may be contaminated or otherwise altered by the probe and therefore should not be used for the assay.

6. **Sample digestion.** Samples are digested using a closed vessel in the MARS microwave system (http://cem.com/page105.html). There are a total of 12 digestion vessels one of which is the control vessel. Each vessel has a spacer at the bottom. Five mL of water is added prior to adding the vials. Three 3 mL vials are placed in each vessel and a cap is put on which has a relief port. The vessel is slid into a sleeve and into the holder in the microwave. Digestion is carried out until the probe in the control sample indicates that it is fully digested.

7. **Assess digestion—Particulates visible?** After the previous step, the sample should be examined for undigested material. The liquid will likely become colored, but particulates should be visible on close examination. If particulates are visible, the previous step should be repeated. If particulates remain, the sample likely contains high mineral content. Therefore, hydrofluoric acid digestion is recommended (not covered in this SOP).

8. **Dilute for ICP-MS analysis.** After being digested the samples are transferred to cleaned (nitric acid leached), tared polypropylene tubes. To transfer, the liquid is poured into the tubes and the vials should be rinsed with a 5 ppb internal standard mix in 2% nitric acid (Seastar, ultra high purity). This wash was added to the vial and the final volume was brought up to 10 mL's. An additional 10x dilution was also made by taking 1 mL of this solution and placing it in another clean tube and bringing this up to 10 mL with the internal standard. Samples were then ready for analysis by ICP-MS.

9. **ICP-MS analysis.** The elemental analyses can nominally be carried out by any ICP-MS. However, higher accuracy will be obtained with a state-of-the-art ICP-MS, such as the Thermo Electron X Series quadrupole ICP-MS that is used at LLNL. A fully quantitative analysis using a linear calibration curve based on known standards is performed. The internal standard corrects for instrument drift and suppression from the digest matrix. K, Fe, As and Se are run in CCT (Collision Cell Technology) mode due to polyatomic interferences. V, Cr and Mn are also run in CCT to confirm values obtained in regular mode that could be affected by high carbon and/or chloride content. Samples are run both concentrated and using the 10x dilution to ensure analytes fall within the calibration range.

10. **Generate quantitative elemental data.** Raw data is reported as ng/g (ppb). The results are corrected back through the 10x dilution (if used) to the 10ml volume of the digested sample and reported in ng (e.g. 10ng/g * 10g = 100ng). Due to the low concentration of many analytes in the spores, the average of the 3 microwave blanks (prepared identically to the samples) is subtracted from the sample concentration. NIST traceable certified reference materials and serial dilutions are analyzed for accuracy. Precision can be established by replicate analyses.

    Absolute concentration can be determined based on the initial mass by dividing the calibrated ng data by the mass of sample in mg, yielding µg/g (ppm). If the sample was not weighed or the weight is deemed to be unreliable, quantification can be performed based the calibrated ng data. Total metal content is calculated by summing the raw data. This sum is used to normalize the measurements for individual elements.

11. **Delta value calculation.** The quantitative elemental data are used to calculate the delta value for the samples to be tested (See accompanying report).

12. **Receiver-Operating Characteristic (ROC) curve analysis.** The data should be compared to the ROC curve for comparable samples (See accompanying report). The data are reported as a likelihood ratio.

Sample Matching SOP References:

1. S.P. Velsko, Bioagent Sample Matching using Elemental Composition Data: an Approach to Validation, a report to the Department of Homeland Security, Lawrence Livermore National Laboratory, April 24, 2006.

2. S.P. Velsko, P.K. Weber, C.E. Ramon, R. Lindvall, M.L. Davisson, M. Robel, Bioagent sample matching using elemental composition data, a draft report to the Department of Homeland Security, Lawrence Livermore National Laboratory, October 29, 2009.

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